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Different regrouping schedules in semi group-housed rabbit does: Effects on agonistic behaviour, stress and lesions

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ABSTRACT

Although group housing of naturally social animals like rabbits (*Oryctolagus cuniculus*) is desirable for ethical reasons, social conflicts can significantly increase the risk for injuries as well as stress incidences and negatively affect their welfare. A common housing system in Switzerland is the "semi group-housing". Here, rabbit does are kept individually shortly before the birth of their kits until 12 days post-partum (pp) after which they are regrouped. Despite positive aspects of this housing system, like the reduction of pseudo pregnancy and crushing of kits, fights between the does often occur after the regrouping process. This study examined whether prolonged postpartum separation may reduce agonistic interactions, stress, and lesions. In total, data were collected over 5 trials on 57 Hycole breeding does. Per trial, three groups of 8 does each were artificially inseminated on day 10 pp and regrouped on either day 12, 18 or 22 pp. Non-pregnant does were replaced in every trial. Agonistic behaviour, anogenital distance, lesions and increased body temperature, as a stress indicator, were documented. The effect of the regrouping treatment on the rate of injury and agonistic interactions was different depending on the trial (lesions: interaction trial x treatment $\chi 2 = 44.21$, df = 8, $P < 0.0001$; agonistic interactions: interaction treatment x trial $\chi 2 = 23.59$, df = 8, $P = 0.003$). During winter trials (November- February), the numbers of lesions and agonistic interactions were generally lower than in the trials during summer. None of the animals with temperature transponders showed a body temperature increase after regrouping ($P = 0.98$), however, there was an increase after the artificial insemination ($P = 0.019$). The anogenital distance, measured during the artificial insemination process, was not correlated to the aggressive behaviour of does ($r = 0.028$; $P = 0.78$). These findings suggest that prolonging single housing only reduced lesions and fighting in some trials but failed to do so in others. Group composition, individuality and season are discussed as relevant factors for the extent of agonistic interactions. Against our expectations, in none of the groups a stress response after regrouping was found according to body temperature measures.

1. Introduction

Domestic rabbits (*Oryctolagus cuniculus*) are gregarious animals, descending from European wild rabbits with large territories and family associated social groups (2–3 bucks, 2–9 does and their offspring) (Surridge et al., 1999). Unfortunately, in commercial production systems, single as well as continuous group housing have shown deficits in meeting all the specific biological needs of these animals. Single housing deprives them of social interactions; a natural condition they demonstrably value. In fact, one study showed that rabbits in groups of two were often observed in physical contact although the size of the cage allowed bodily separation (Chu et al., 2004). In a social motivation study, rabbits worked almost as hard for limited body contact as they did for food, emphasizing the importance of social contact (Seaman et al., 2008). Furthermore, stereotypies and self-destructive behaviours (bar or hair- chewing) (Gunn and Morton, 1995), limited locomotion possibilities (Chu et al., 2004) as well as frustration and boredom can result from the single housing system. Continuous group housing, however, led to agonistic interactions and lesions (Andrist et al., 2013), low productivity (Mugnai et al., 2009) and high kit mortality (Szendrő et al., 2016).

Semi-group housing, where does are separated during the most critical phase shortly before parturition until at least 12 days afterwards, has shown to have many benefits in comparison to both single and continuous group housing systems. In fact, due to temporary separation, double litters in one nest box at birth are avoided, as well as

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infanticides, as these are mostly restricted to the first 10 days after parturition (Rödel et al., 2008). After regrouping, the animals benefit from social contact and the possibility for increased locomotion behaviour. Additionally, some studies have shown promising results concerning production and reproductive performance in this system: better feed intake and weight gain for kits (Machado et al., 2016) and similar fertility rates and litter size (Maertens et al., 2011; Maertens and Buijs, 2015) compared to single housing. Nonetheless, the occurrence of lesions and fights between the animals remains a problem, especially just after regrouping (Andrist et al., 2013; Rommers et al., 2006; Valuska and Mench, 2013).

The aggressive behaviour is triggered by the separation as well as the reintroduction of new does, as sick and non-reproducing animals are constantly removed and replaced (Andrist et al., 2012). This causes a regular change in the hierarchy of the groups, accompanied by agonistic behaviour (Graf et al., 2011; Ruis, 2006) and stress responses (Holst et al., 1999). Nonetheless, fights about hierarchy may not be the only cause of agonistic interactions among the does. Though rabbits only nurse their litter about once or twice a day for about 3 − 5 min (González-Mariscal et al., 2007; Rödel et al., 2008), they are known for fierce offspring defence, not only against predators, but also towards conspecific intruders (Rödel et al., 2008; Schlolaut et al., 2013). Naturally, rabbit does wean their kits between 20–28 days of age, depending on their current pregnancy state (Hudson et al., 1996, 2000). Non-pregnant does wean their offspring at 5–6 weeks after kindling (Trocino and Xiccato, 2006). Furthermore, studies have shown that, although infanticides mostly happen until 10 days postpartum (dpp), does can still react defensively when their burrows are approached up to 20 days of lactation (Rödel et al., 2008). Zomeño et al. (2017) also considered the age of the kits and stated that aggression among females may be reduced with older kits already starting to leave the nest box area. Accordingly, regrouping of does after 12 dpp may be too early in the current semi-grouping system in Switzerland, as the does' drive to defend their kits increases their aggressiveness towards others. Hence, the aim of this study was to examine whether a different time schedule of regrouping would change the level of agonistic interactions and associated lesions. According to our hypothesis, a regrouping at a later lactation time point with older kits should result in less lesions, stress and agonistic interactions.

Additionally, another way of reducing aggression in group-housed does could be identifying and selecting less aggressive does. For instance, anogenital distance (AGD) (= distance from base of genital papilla to centre of the anal opening (Bánszegi et al., 2012; Dušek and Bartoš, 2012)) is influenced by intrauterine testosterone exposure (Bánszegi et al., 2010) and has been correlated with the aggressiveness of does in the past (Buijs et al., 2016): does with a greater anogenital distance at birth showed more offensive agonistic behaviour upon grouping as adults. However to our knowledge, no studies have analysed if this association is still detectable when AGD is only measured in adult does. Therefore we recorded AGD and evaluated its influence as a co-variate in the agonistic interactions' analysis.

2. Animals, material & methods

2.1. Animals and housing

A total of 57 multiparous Hycole rabbit does over 18 weeks of age were used in this study. They were kept in a semi-group housing system on a commercial rabbit farm in Switzerland according to a Swiss label program for animal-friendly husbandry (BTS WWW Document, 2019). During the single housing phase, which started at 1 day before parturition, the cage space per animal was 1.6 m^2, including a nest box (0.30 m × 0.40 m) and a platform. In the group phase afterwards, all 8 cages per pen were opened simultaneously at the top and the animals could reunite in a common floor area (3.20 m × 2.20 m) covered with straw (Fig. 1). The rabbits had *ad libitum* access to water, hay and commercial rabbit pellets (UFA 925, UFA AG, Herzogenbuchsee, Switzerland). They were kept in a standard procedure on a 41-day reproductive cycle under natural daylight conditions and were artificially inseminated (AI) on day 10 pp. Five days before and two days after AI, an artificial light regime was put in place with a 16 h light phase period.

Fig. 1. Pen outlay: Individual cage (1), closable grid with hay on top (2), extractable nest box (3), platform (4), individual pellet dispenser (5), common floor area (6).

2.2. General procedure and treatments

After AI, three different treatments were applied: the first treatment group (TG12) was group-housed at 12 days postpartum (dpp), the second one (TG18) at 18 dpp and the third treatment group (TG22) at 22 dpp. Each group consisted of 8 does. All 3 treatments were applied during 5 trials (August 2018 to March 2019). A trial lasted from 1 day before parturition until the weaning of the kits (25 dpp). After each trial, the groups switched randomly to a different treatment. Non-pregnant does were replaced by pregnant ones before each new trial, as is common practice on farms. This resulted in the sample size of 57 animals. However, there were not enough pregnant does due to a management problem in trials 2 and 3. In these cases, the groups were supplemented with non-pregnant does to keep the group size constant at 8 does per pen to avoid a reduction of agonistic interactions due to smaller groups (Buijs et al., 2016; Zomeño et al., 2017). However, the does without kits were not included in the analyses. To facilitate individual monitoring, all animals were marked with ear tags and livestock spray before each trial. Barn temperature and relative air humidity were measured throughout the experiment using the HOBO® Datalogger U10 − 003 (Onset Computer Corp., Bourne, MA 02,532) and ranged from 8.4 °C–20.1 °C and 26 %–67 %, respectively.

2.3. Lesions

Before regrouping (8 dpp), all animals were examined for lesions in order to exclude pre-existing wounds in the later dataset. Fights between does normally occur during the first 2–3 days after regrouping (Albonetti et al., 1990 in Rommers et al., 2011; Graf et al., 2011), however, new lesions are still detected after 5 days (Andrist et al., 2014). Therefore, we assessed lesions individually for each animal 6 days after regrouping. For scoring, we used a tagged visual analogue scale, based on the modified score of Andrist et al. (2012): 0 = no

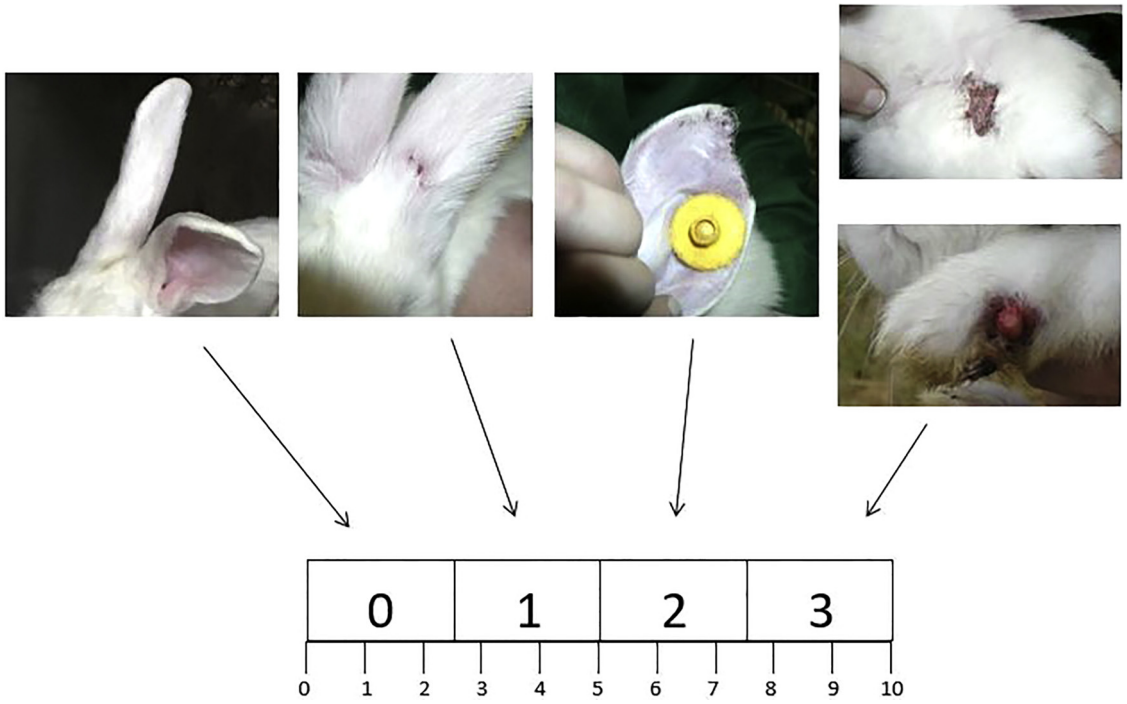

Fig. 2. A tagged visual analogue scale (modified from Andrist et al., 2012) to score the occurrence and severity of lesions on the body of rabbit does.

lesions; 1 = abrasions or superficial lesions in the dermis smaller than 1 cm^2; 2 = deeper lesions in the connective tissue or lesions larger than 1 cm^2; 3 = very deep lesions in muscle tissue and/or or visible bone structure (Fig. 2).

Wounds in the eye and genital area were scored higher due to assumed increased painfulness based on their location (Varga, 2014). One person, blinded to the design of the study and treatment group of the animals, scored the wounds. For this visual inspection, the doe was held in one person's lap, while the evaluator gently inspected the ears, eyes, nose, neck, and back. Afterwards, the animal was turned on its back and the teats, the genital area, as well as the legs and paws were examined. The procedure always followed this exact order. Lesions clearly attributable to pododermatitis were disregarded, as they were not caused by aggressive behaviour. The location, number and highest severity score of all lesions were recorded per animal. No pathology was performed on deceased animals (N = 3). However, they were inspected for obvious external causes of death by a trained veterinarian. The kits of these does were equally distributed in the treatment group.

2.4. Behavioural observations

Twenty-four-hour video recordings were made immediately after regrouping, which took place at 7 a.m. at the respective days. All does were observed from the videos as focal animals. The agonistic behaviour was coded during the first 2 h after regrouping and during 3–5 a.m. the next day. These times were chosen based on a prior scanning of the videos for the phases with the most agonistic interactions after regrouping. Furthermore, it has been reported that most agonistic interactions happen immediately after regrouping (Andrist et al., 2013; Rommers et al., 2011). The behaviour was coded with a modified version of Graf's ethogram (Andrist et al., 2012; Graf et al., 2011; Zomeño et al., 2018); Table 1). The number and location ("platform", "home cage", "foreign cage", "common floor area"; see Fig. 1) for each agonistic behaviour were documented for the animal who started the interaction. The coder was blinded to the treatment groups.

Table 1
Agonistic behaviour of does (based on ethogram of Graf et al., 2011).

Behaviour	Description of behaviour
Biting	Gripping with the teeth
Boxing	Hitting with the front paws
Chasing	Aggressive following of another individual for at least three jumps
Carousel fights	Rapid circling with the opponent's rear end gripped between the teeth
Threatening	Quick head movement towards another doe
Attacking	Abruptly running towards a groupmate
Ripping	Two does kicking each other with hind legs
Mounting	Approaching another individual from behind and positioning over the back

2.5. Body temperature

Measurements of subcutaneous body temperature, an estimate of core temperature (Chen and White, 2006), were used as indicators of stress (Dallmann et al., 2006; Snow and Horita, 1982). The normal core body temperature in rabbits ranges between 38.5 and 40.0 °C (Graf et al., 2011). To avoid inducing stress by handling or approaching the animals, a transponder system was used: Before the start of each trial, an RFID implant (Plexx B.V., 6660 AB Elst, the Netherlands) was injected laterally under the cervical skin of one randomly selected animal per treatment. For logistical reasons, the body temperature could be measured only in one animal for each trial x treatment combination. Tagged animals that became pregnant again were reinstated in the next trial in another treatment group for refinement purposes (N = 11). In total 4 does were used twice. The readers (BMDS® DAS-6001, Plexx) were installed in the cages of the affected animals next to the food dispenser. Body temperature was recorded throughout an entire trial. The animals were regularly health checked to exclude a temperature increase due to infection. For individual stress detection, the median body temperature of the 4 h period following regrouping ("after regrouping") was compared to the median body temperature during the same 4 h period one day prior to regrouping ("before regrouping"). The same 4 h comparison was made between the temperature before and

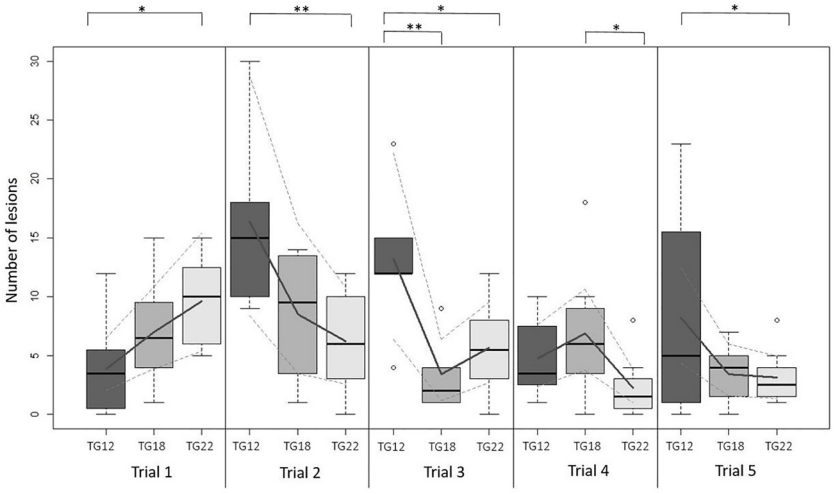

Fig. 3. Number of lesions in the treatment groups (TG12, TG18, TG22) and trials (1- 5). The box includes the number of occurring lesions in the respective trial and treatment interaction, the horizontal line represents the median, the significance levels are represented as followed: * = P < 0.01; ** = P < 0.001; *** = P < 0.0001. Solid line = model estimates. Dash lines = 95 % confidence intervals.

after the artificial insemination ("before AI", "after AI") to avoid the influence of circadian and individual temperature changes (Jilge et al., 2001). As body temperature in pregnant animals naturally starts to decrease around 11 days after insemination (Jilge et al., 2001; own results), we could not interpret body temperatures after 21 dpp, which involved the week after regrouping for TG22. Therefore, we examined the weekly change after regrouping only for treatments TG12 and TG18.

2.6. Anogenital distance

AGD was recorded during AI for management and refinement purposes, as the animals were then already situated in an appropriate position for measurements. The AGD of all the does was measured from the base of the genital papilla to the centre of the anal opening (Bánszegi et al., 2012; Dušek and Bartoš, 2012), using a digital calliper. The mean of three consecutive measurements per doe by the same person was used to improve accuracy.

2.7. Statistical evaluation

The data were evaluated using the statistics program R (R Core Team, 2019). The number of agonistic interactions and the number of lesions were analysed using generalized linear mixed models. For the lesion severity score a linear mixed model was applied. In all models the full model included trial and treatment and their interaction as fixed factors and due to repeated measures, doe ID as a random term. A linear mixed model was applied for body temperature including event (4 levels: "before AI", "after AI", "before regrouping", "after regrouping ") as fixed and doe ID and trial as random factors. Additionally, anogenital distance was initially considered as a co-variate to investigate its relationship with agonistic behaviour, but was excluded from the final model due to non-significant effect. Body weight was not included as fixed effect due to lack of variance between the does. The residuals of these models were graphically tested for normality and homogeneity of variance, using DHARMa plots (Hartig, 2019). Lesion severity score was normally distributed. The number of agonistic interactions and the numbers of lesions were Poisson distributed. Due to overdispersion, the analysing model for number of lesions was transformed using negative binomial link function. All calculations were performed using the lme4 package by Bates et al., 2019. The model selection was done by comparing the full model with the intercept-only model and if significant the full model was compared to effects model excluding the interaction term. If the treatment x trial interactions were significant, we used Bonferroni-corrected planned post hoc tests for multiple comparisons ("multcomp" package by Hothorn et al., 2019). Estimates and confidence intervals of the models were calculated using the boot package by Canty and Ripley, 2019 and the pbkrtest package by Højsgaard, 2017. The correlation between AGD, body weight and number of agonistic interactions were analysed by Spearman rank correlation tests. Repeatability for lesion scoring and anogenital measurements was calculated using the "rptR" package published by Stoffel et al., 2019. All means are given with ± SD. Three animals died prior to the end of their particular trial and were excluded from the analysis due to missing values.

2.8. Ethical approval

This study was approved by the Cantonal Office of Aargau (No. 30,611) and met all cantonal and federal regulations on animal experimentation.

3. Results

3.1. Lesions

3.1.1. Number and location of lesions

The inter-observer (r = 0.913; 97.5 % CI (0.85 to 0.95); P < 0.0001) as well as the intra-observer repeatability (r = 0.98; 97.5 % CI (0.98 to 0.99); P < 0.0001) for the lesion scoring were considered to be very good. The number of lesions between the treatment groups varied strongly depending on the trial (interaction trial x treatment: $\chi 2 = 44.21$, df = 8, P < 0.0001). Thus, in contrast to our hypothesis, the animals in TG22 showed significantly more lesions than animals in TG12 during the first trial. In the second and third trials however, TG12 had significantly more lesions compared to TG18 and TG22. In the fourth trial, TG18 had a higher injury rate than TG22. In trial 5 there were again more lesions in TG12 compared to TG22 (Fig. 3).

In general, there were fewer lesions in the trials during November-March than in the trials during August-October. Most lesions occurred on the lower back area (34.7 %), hind legs (18.7 %) and ears (10.5 %) of the animals. The three deceased animals showed no external wounds which would indicate lesions as the cause of death.

3.1.2. Severity score

Altogether only 17.8 % (N = 18) of the does were without lesions. More than half (59.4 %) (N = 60) had mild lesions (score 1), 19.8 % (N = 20) had moderate (score 2) and 3 % (N = 3) had severe lesions (score 3). Fig. 4 shows the detailed distribution of the severity score between the three treatment groups. There was a trend for an interaction between treatment x trial ($\chi 2 = 16.97$, df = 8, P = 0.08). Overall lesion severity decreased from summer to winter trials ($\chi 2 = 13.93$,

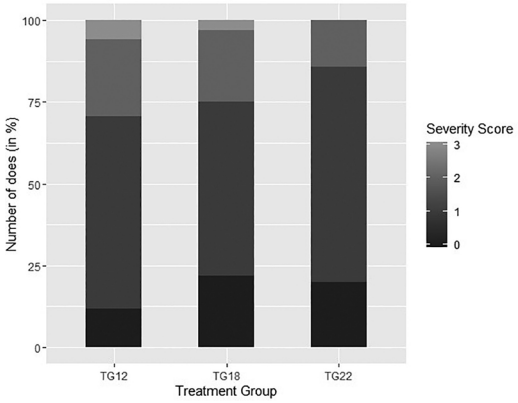

Fig. 4. Distribution of severity score for lesions in treatment groups (TG12, TG18, TG22) given in percentage (%) (N = 34 for TG12, N = 32 for TG18, N = 35 for TG22).

df = 4, P = 0.007), however there was no significant difference found between the treatment groups ($\chi 2 = 2.96$, df = 2, P = 0.228).

3.2. Number and location of agonistic interactions

Overall, about half of the agonistic interactions took place in the own nest of the initiating animal (48.5 %). Independent of treatment group, the most common interactions were "attacking" (23.02 %), "chasing" (18.35 %), "threatening" (16.47 %) and "biting" (16.17 %). "Mounting" did not occur. The number of agonistic interactions an animal started was weakly correlated with its number of lesions (r = 0.41; P < 0.0001) and, like the lesions, it depended on an interaction between trial and treatment ($\chi 2 = 23.59$, df = 8, P = 0.003). In the first trial, TG18 and TG22 showed more agonistic behaviour than TG12. In trial 2, the opposite was the case; TG12 displayed more agonistic behaviour compared to TG18 and TG22. The same applied to trial 3 and 4. However, in trial 5, there was no significant effect between the treatment groups (Fig. 5).

3.3. Anogenital distance

The repeatability for the AGD was good (r = 0.77; 97.5 % CI (0.59 to 0.88); P < 0.0001). The anogenital distance was not correlated to the number of agonistic interactions (r = 0.028; P = 0.78). It was however positively correlated with body weight (r = 0.24; P = 0.01). Moreover, during the trials in August-October, AGDs were significantly

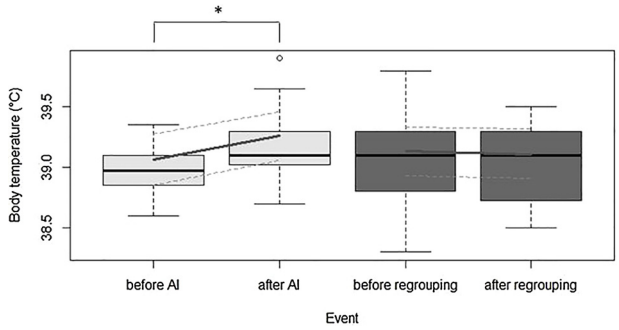

Fig. 6. Comparison of median body temperature of all animals (N = 11) 4 h after artificial insemination (AI) and the same 4 h the day before and between 4 h after regrouping and the same 4 h the day before. The significance levels are represented as followed: * = P < 0.01; ** = P < 0.001; *** = P < 0.0001. Solid line = model estimates. Dash lines = 95 % confidence intervals.

larger than during winter trials (Wilcoxon signed-rank test: P = 0.002) in repeatedly measured animals.

3.4. Body temperature

The event ("before AI", "after AI", "before regrouping", "after regrouping") had a significant effect on body temperature of the does ($\chi 2 = 9.039$, df = 3, P = 0.029): While body temperatures before and after regrouping did not differ (P = 0.98, median temperature change = 0.03 °C ± 0.3) in any of the treatments, body temperatures increased after AI (P = 0.019, median temperature change = 0.19 ± 0.21) (Fig. 6). No significant differences in body temperature were found over the course of one week for TG12 and TG18.

4. Discussion

4.1. Lesions and agonistic interactions

The interaction between the effects of time point of regrouping and trial was found for the number of lesions and agonistic interactions in this study. While in trial 1, TG12 showed the lowest number of lesions and agonistic interactions, the opposite was true for the three following trials: a decrease in agonistic interactions and lesions in TG18 and TG22 compared to TG12. As the does were multiparous, we cannot exclude that the does in TG12 during trial 1 were more familiar with each other than in the other two groups and therefore happened to show a very low level of aggressive behaviour (Crowell-Davis, 2007). However, all

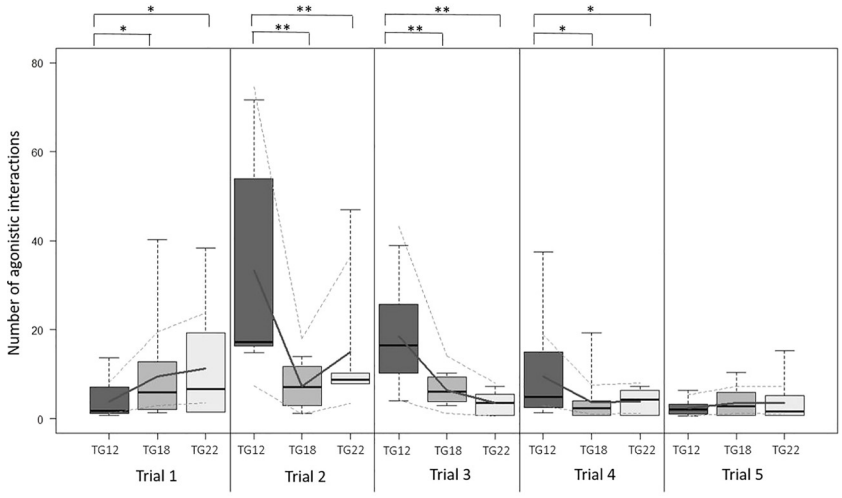

Fig. 5. Number of agonistic interactions in the treatment groups (TG12, TG18, TG22) and trials (1-5). For details see Fig.3.

groups were formed completely randomly in the beginning and between two and five does were exchanged in all groups in the following trials. This indicates that the composition of the group and thus the social dynamics might be very important for the level of agonistic interactions during and after regrouping. Moreover, there was a high level of individual differences between the does. While some started most of the interactions (up to 75 times), others displayed very little aggressive behaviour towards others (0–1 times) during the recorded period. Additionally, trials 4 and 5 showed no differences between the treatment groups concerning lesions and fights, respectively. This may be caused by the fact that all the treatment groups in these two winter trials had an overall very low incidence of lesions and agonistic interactions.

Considering the severity of lesions, TG22 was the only treatment group without score 3, although this score had an overall low incidence with only three animals affected in a total of five trials and severity score was not influenced by the treatment. Other studies showed a decrease of severity of lesions with longer periods of separation, however they compared regrouping before (-8 days) and after parturition (+18 days). Accordingly, the animals were then already familiar with each other on day 18 (Zomeño et al., 2017, 2018). Group stability may have played an important role in the reduction of agonistic interactions, as found in another study (Andrist et al., 2012). In fact, another study with regrouping at 18 dpp with non-familiar does had a very high incidence of lesions (Rommers et al., 2014). However, comparisons with previous studies are difficult due to differing group sizes and overall management. In general, nest defence could have been a likely cause for agonistic interactions in our study, which was evident by the high number of fights in the own nest box area compared to different locations. Nonetheless, hierarchy formation after separation remained important: Every treatment group showed agonistic behaviour at regrouping and the day after. However, a significant decrease in agonistic interactions was observed after only 6 days in all treatment groups (Munari et al., in press). This corresponds to earlier studies in which the animals reduced fighting after establishing a hierarchy during the first 2–10 days after regrouping (Mykytowycz, 1958; Rommers et al., 2011). The main location of the lesions ("hind legs" and "lower back") can be explained by the most frequent aggressive actions of chasing and carousel fighting when the attacker was running behind the fleeing animal. Furthermore, there were generally more lesions and more frequent agonistic behaviour in the summer than in the winter trials. A study by Andrist et al. (2013) also showed an increase of lesions in the summer season. This may be caused by an increased sexual activity during this period (Rommers et al., 2006; Southern, 2019).

To investigate this point, hormone concentrations (testosterone, estradiol and progesterone) from blood samples collected during this experiment were analysed (Braconnier et al., in prep). Additionally, possible behavioural differences between the physiological states "pregnant-lactating" and "non-pregnant-lactating" have to be taken into account, as their hormonal background has been reported to be significantly different (González-Mariscal et al., 2009, 2016; Hoffman et al., 2009; Rosenblatt, 1980).

4.2. Body temperature

We did not detect an increase in body temperature in any of the treatment groups after regrouping in comparison to the individual basal temperature the day before. However after AI, body temperatures rose. To our knowledge, studies have not found an increase in temperature due to ovulation (possible after AI) in rabbit does (Bahat et al., 2005; Tsutsumi et al., 1967), therefore we exclude ovulation per se as the cause of the rise in temperature. Additionally, as AI requires the chasing and catching of the rabbit and putting it head down into a tube with constraint, we assume that our methodology using RFID implants was able to detect severe acute stress.

We interpret the lack of a rise in body temperature after regrouping as a sign that the imposed stress was less severe than the stress response at AI. A possible explanation could be that the animals in all groups had enough space for escaping, avoiding confrontation and hiding purposes in comparison to other studies with a more restricted area (Rommers et al., 2014). Even regrouping, involving agonistic interactions, might cause less stress than initially expected. In fact, Graf et al. (2011) neither found any changes in body temperature up to three days after regrouping in a similar setting as ours (home pen and replacing non-pregnant does). The increase in body temperature in the experiment of Andrist et al. (2014) was possibly not due to stress, as the basal temperature value for his rabbit does was measured shortly before parturition. During this time period, the body temperature is naturally lower than on the days afterwards (Jilge et al., 2001; own results).

In general, we assume that AI caused a significantly greater stress response than regrouping. The possible explanation for this may be in the biology of rabbits. While fighting for nesting sites and ranking order is a natural behaviour for these animals (Albonetti et al., 1990 in DiVincenti et al., 2016), the unfamiliar handling before the insemination process can be interpreted as an attack by predators and lead to a reaction of fear, like freezing and trying to escape (personal observations). Although baseline body temperature was restored in all animals after a few hours, the stress caused by this process should be considered in production and further studies, in regards to welfare, but also to low fertility results (Braconnier et al., in press; Mugnai et al., 2009). However, our sample size was very small (N = 11), with only one focal animal per treatment in each trial, so no generalized conclusion for all breeding does can be drawn. In fact, reacting and coping with stress can vary greatly between individuals (Carere et al., 2010; Koolhaas, 2008). Also studies have found, that coping success is not only influenced by genetics and management, but experience as well (*i.e.* the effects of learning) (Daniewski and Jezierski, 2003; Verga et al., 2007). We only worked with multiparous does, familiar to the used husbandry and system, so this might have influenced the outcome, as well.

4.3. Anogenital distance

Though all animals were in a similar parity and the intra-observer repeatability was good, we could not detect a correlation between the level of agonistic behaviour and the AGD. As we found significant seasonal differences in repeatedly used and measured animals, we assume the parameter may underlie more variation than previously expected. In fact, one study showed changing AGD sizes during a single oestrus cycle in mice and indicated prenatal androgens may not have such a robust effect on female genital morphology as previously thought (Dušek and Bartoš, 2012). As rabbits are induced ovulators, they have no regular oestrous cycle. They show however periods of receptivity, detectable by a swollen, pink-purple or reddish-purple coloured vulva (O'Malley, 2005; Paré and Paul-Murphy, 2003). This swelling may be an explanation for the variation in measurements (Kerkouche et al., 2014) specifically for the overall decrease of AGD during winter trials, as ovarian activity simultaneously decreases with the decreasing photoperiod (O'Malley, 2005; Vella and Donnelly, 2012). Hence, selecting less aggressive rabbit does based on their AGD may be possible before they reach sexual maturity (Buijs et al., 2016), but afterwards may be subjected to too much variation due to the receptivity induced fluctuations of vulva size. Comparing our results to other studies is difficult because the investigated agonistic behaviour, which was related to the AGD, was often very specific ("chin marking" (Bánszegi et al., 2010) or "tail rattling" in mice (Palanza et al., 1995)). The correlation between AGD and body weight has been shown in a study before (Buijs et al., 2016), but in another study (Bánszegi et al., 2012), this correlation was absent.

4.4. Future outlook

The aggressive behaviour in rabbit does remains a complex and

multifactorial problem. Future research should focus more on the individuality of these animals as well as on possible genetic variation in social behaviour, like it has been done in other farm species like chickens and pigs (Jensen, 2006; Turner, 2011).

Enlarging space (Mykytowycz, 1958; Rommers et al., 2006) as well as pre-kindling training to use a specific nest (Mugnai et al., 2009) have shown promising results in reducing aggressive behaviour, but are difficult to apply on farms for practicability and financial reasons. Thus, other more easily applicable and cheap interventions to increase the welfare of these animals might be tested such as group stability (Andrist et al., 2012; Holst et al., 2002), reduced group size (Buijs et al., 2016; Zomeño et al., 2017), use of a familiar pen (Graf et al., 2011; Rommers et al., 2014), or close monitoring and removing of non-compatible aggressors (Morton et al., 1993). Specifically considering the last aspect, precision livestock farming ("PLF") may also serve as an important tool in the future for identification purposes (Norton et al., 2019). Additionally, we require research able to efficiently disentangle seasonal, hormonal and group composition effects as these appear to have a crucial influence on the varying levels of aggressiveness in breeding does.

Finally, as research has mainly focused on negative social behaviour, the benefits from positive interactions between the animals are often overlooked or poorly understood. Therefore, the aspects of prosocial interactions, like allogrooming and lying in bodily contact, need to be targeted more in future studies, as they are crucial to the health and well-being of domestic animals (Rault, 2019). Only then, we are able to develop a cost-benefit analysis of group-housing of breeding rabbit does.

5. Conclusions

Prolonging single housing after day 12 pp in semi-group housing reduced aggressive interactions and lesions in some trials but failed to do so in others. Besides the identity and composition of the group, the season seemed to be relevant for the extent of agonistic interactions. Contrary to expectations, no elevated stress levels were detected after regrouping.

Declaration of Competing Interest

The authors declare no conflict of interest.

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References

Andrist, C.A., Bigler, L.M., Würbel, H., Roth, B.A., 2012. Effects of group stability on aggression, stress and injuries in breeding rabbits. Appl. Anim. Behav. Sci. 142, 182–188. https://doi.org/10.1016/j.applanim.2012.10.017.

Andrist, C.A., van den Borne, B.H.P., Bigler, L.M., Buchwalder, T., Roth, B.A., 2013. Epidemiologic survey in Swiss group-housed breeding rabbits: extent of lesions and potential risk factors. Prev. Vet. Med. 108, 218–224. https://doi.org/10.1016/j.prevetmed.2012.07.015.

Andrist, C.A., Bigler, L., Würbel, H., Roth, B.A., 2014. Masking odour when regrouping rabbit does : Effect on aggression, stress and lesions. Livest. Sci. 170, 150–157. https://doi.org/10.1016/j.livsci.2014.10.017.

Bahat, A., Eisenbach, M., Tur-Kaspa, I., 2005. Periovulatory increase in temperature difference within the rabbit oviduct. Hum. Reprod. 20, 2118–2121. https://doi.org/10.1093/humrep/dei006.

Bánszegi, O., Altbäcker, V., Dúcs, A., Bilkó, Á., 2010. Testosterone treatment of pregnant rabbits affects sexual development of their daughters. Physiol. Behav. 101, 422–427. https://doi.org/10.1016/j.physbeh.2010.07.020.

Bánszegi, O., Szenczi, P., Dombay, K., Bilkó, Á., Altbäcker, V., 2012. Anogenital distance as a predictor of attractiveness, litter size and sex ratio of rabbit does. Physiol. Behav. 105, 1226–1230. https://doi.org/10.1016/j.physbeh.2012.01.002.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2019. lme4: Linear Mixed-effects Models Using "Eigen" and S4. https://cran.r-project.org/package=lme4.

BTS [WWW Document], 2019.. https://www.blw.admin.ch/blw/de/home/instrumente/direktzahlungen/produktionssystembeitraege/tierwohlbeitraege.html.

Buijs, S., Vangeyte, J., Tuyttens, F.A.M., 2016. Effects of communal rearing and group size on breeding rabbits' post-grouping behaviour and its relation to ano-genital distance. Appl. Anim. Behav. Sci. 182, 53–60. https://doi.org/10.1016/j.applanim.2016.06.005.

Canty, A., Ripley, B., 2019. boot: Bootstrap Functions (Originally by Angelo Canty for S). https://cran.r-project.org/package=boot.

Carere, C., Caramaschi, D., Fawcett, T.W., 2010. Covariation between personalities and individual differences in coping with stress: converging evidence and hypotheses. Curr. Zool. 56, 728–740. https://doi.org/10.1093/czoolo/56.6.728.

Chen, P.H., White, C.E., 2006. Comparison of rectal, microchip transponder, and infrared thermometry techniques for obtaining body temperature in the laboratory rabbit *(Oryctolagus cuniculus)*. J. Am. Assoc. Lab. Anim. Sci. 45, 57–63.

Chu, L.R., Garner, J.P., Mench, J.A., 2004. A behavioral comparison of New Zealand White rabbits *(Oryctolagus cuniculus)* housed individually or in pairs in conventional laboratory cages. Appl. Anim. Behav. Sci. 85, 121–139. https://doi.org/10.1016/j.applanim.2003.09.011.

Crowell-Davis, S.L., 2007. Behavior problems in pet rabbits. J. Exot. Pet Med. 16, 38–44. https://doi.org/10.1053/j.jepm.2006.11.022.

Dallmann, R., Steinlechner, S., Von Hörsten, S., Karl, T., 2006. Stress-induced hyperthermia in the rat: comparison of classical and novel recording methods. Lab. Anim. 40, 186–193. https://doi.org/10.1258/002367706776319015.

Daniewski, W., Jezierski, T., 2003. Effectiveness of divergent selection for open-field activity in rabbits and correlated response for body weight and fertility. Behav. Genet. 33, 337–345. https://doi.org/10.1023/A:1023454726734.

DiVincenti, L., Rehrig, A.N., Rehrig, A.N., 2016. The social nature of european rabbits *(Oryctolagus cuniculus)*. J. Am. Assoc. Lab. Anim. Sci.

Dušek, A., Bartoš, L., 2012. Variation in Ano-Genital Distance in Spontaneously Cycling Female Mice. Reprod. Domest. Anim. 47, 984–987. https://doi.org/10.1111/j.1439-0531.2012.02003.x.

González-Mariscal, G., McNitt, J.I., Lukefahr, S.D., 2007. Maternal care of rabbits in the lab and on the farm: endocrine regulation of behavior and productivity. Horm. Behav. 52, 86–91. https://doi.org/10.1016/j.yhbeh.2007.03.028.

González-Mariscal, G., Gallegos, J.A., Sierra-Ramirez, A., Flores, J.G., 2009. Impact of concurrent pregnancy and lactation on maternal nest- building, estradiol and progesterone concentrations in rabbits. World Rabbit. Sci. 17, 145–152. https://doi.org/10.4995/wrs.2009.654.

González-Mariscal, G., Caba, M., Martínez-Gómez, M., Bautista, A., Hudson, R., 2016. Mothers and offspring: the rabbit as a model system in the study of mammalian maternal behavior and sibling interactions. Horm. Behav. 77, 30–41. https://doi.org/10.1016/j.yhbeh.2015.05.011.

Graf, S., Bigler, L., Failing, K., Würbel, H., Buchwalder, T., 2011. Regrouping rabbit does in a familiar or novel pen: Effects on agonistic behaviour, injuries and core body temperature. Appl. Anim. Behav. Sci. 135, 121–127. https://doi.org/10.1016/j.applanim.2011.10.009.

Gunn, D., Morton, D.B., 1995. Inventory of the behaviour of New Zealand White rabbits in laboratory cages. Appl. Anim. Behav. Sci. 45, 277–292. https://doi.org/10.1016/0168-1591(95)00627-5.

Hartig, F., 2019. DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. https://cran.r-project.org/package=DHARMa.

Højsgaard, U.H.S., 2017. pbkrtest: Parametric Bootstrap and Kenward Roger Based Methods for Mixed Model Comparison. https://cran.r-project.org/package=pbkrtest.

Hoffman, K.L., Martínez-Alvarez, E., Rueda-Morales, R.I., 2009. The inhibition of female rabbit sexual behavior by progesterone: progesterone receptor-dependent and-independent effects. Horm. Behav. 55, 84–92. https://doi.org/10.1016/j.yhbeh.2008.08.011.

Holst, Dvon, Hutzelmeyer, H., Kaetzke, P., Khaschei, M., Schönheiter, R., 1999. Social rank, stress, fitness, and life expectancy in wild rabbits. Naturwissenschaften 86, 388–393. https://doi.org/10.1007/s001140050638.

Holst, Dvon, Hutzelmeyer, H., Kaetzke, P., Khaschei, M., Rödel, H.G., 2002. Social rank, fecundity and lifetime reproductive success in wild European rabbits. Oryctolagus cuniculus 245–254. https://doi.org/10.1007/s00265-001-0427-1.

Hothorn, T., Bretz, F., Westfall, P., 2019. multcomp: Simultaneous Inference in General Parametric Models. https://cran.r-project.org/package=multcomp.

Hudson, R., Shaal, B., Bilkó, Á., Altbäcker, V., 1996. Just three minutes a day: the behaviour of young rabbits viewed in the context of limited maternal care. In: Proc. 6th World Rabbit Congress. 1996 July, Toulouse, France. pp. 395–403. https://doi.org/10.1111/j.1439-0310.2007.01447.x. 2.

Hudson, R., Shaal, B., Martínez-Gómez, M., Distel, H., 2000. Mother–young relations in the European rabbit: physiological and behavioural locks and keys. World Rabbit. Sci. 8, 85–90.

Jensen, P., 2006. Domestication - From behaviour to genes and back again. Appl. Anim. Behav. Sci. 97, 3–15. https://doi.org/10.1016/j.applanim.2005.11.015.

Jilge, B., Kuhnt, B., Landerer, W., Rest, S., 2001. Circadian Temperature Rhythms in Rabbit Pups and in Their Does. pp. 364–373.

Kerkouche, T.N., Zitouni, G.H., Boumahdi, Z., Berbar, A., Kerkouche, R., 2014. Etude Des

Relations Entre Distance Ano-génitale, Parité Et Quelques Caractéristiques De La Reproduction De La Lapine. Livestock Research for Rural Development 26. http://www.lrrd.org/lrrd26/2/kerk26033.html.

Koolhaas, J.M., 2008. Coping style and immunity in animals: making sense of individual variation. Brain Behav. Immun. 22, 662–667. https://doi.org/10.1016/j.bbi.2007.11.006.

Machado, L.C., Martinez-Paredes, E., Cervera, C.C., Paragliola, F., 2016. Performance of rabbit kits originating from collective and individual cages. In: Proceedings 11 Th World Rabbit Congress, pp. 637–651.

Maertens, L., Buijs, S., 2015. Production performances of semi-group housed rabbit does. In: In Proceedings of the 19th International Symposium on Housing and Diseases of Rabbits, Furproviding Animals and Pet Animals. Celle, Germany. pp. 22–31.

Maertens, L., Rommers, J., Jacquet, M., 2011. Le logement des lapins en parcs, une alternative pour les cages classiques dans un système “duo”? [Combi-park housing of females, an alternative for the classical cages when using the “duo” management system?]. 14èmes Journées de la Recherche Cunicole 85–88.

Morton, D., Jennings, M., Batchelor, G., Bell, D., Birke, L., Davies, K., Eveleigh, J., Gunn, D., Heath, M., Howard, B., Koder, P., Phillips, J., Poole, T., Sainsbury, A., Sales, D.D.S., Stauffacher, M., Turner, R., 1993. Refinements in rabbit husbandry. Lab. Anim. 27, 301–329.

Mugnai, C., Dal Bosco, A., Castellini, C., 2009. Effect of different rearing systems and pre-kindling handling on behaviour and performance of rabbit does. Appl. Anim. Behav. Sci. 118, 91–100. https://doi.org/10.1016/j.applanim.2009.02.007.

Mykytowycz, R., 1958. Social behaviour of an experimental colony of wild rabbits, *Oryctolagus cuniculus (L.)* I. Establishment of the colony. CSIRO Wildlife Research 3 (1), 7–25. https://doi.org/10.1071/CWR9580007.

Norton, T., Chen, C., Larsen, M.L.V., Berckmans, D., 2019. Review: precision livestock farming: building “digital representations” to bring the animals closer to the farmer. Animal 1–9. https://doi.org/10.1017/S175173111900199X.

O’Malley, B., 2005. Rabbits. In: O’Malley, B. (Ed.), Clinical Anatomy and Physiology of Exotic Species. Elsevier, New York, pp. 173–195. https://doi.org/10.1016/B978-070202782-6.50011-9.

Palanza, P., Parmigiani, S., Vom Saal, F.S., 1995. Urine marking and maternal aggression of wild female mice in relation to anogenital distance at birth. Physiol. Behav. 58, 827–835. https://doi.org/10.1016/0031-9384(95)00107-T.

Paré, J.A., Paul-Murphy, J., 2003. Disorders of the Reproductive and Urinary Systems. Ferrets, Rabbits and Rodents: Clinical Medicine and Surgery. pp. 183–193. https://doi.org/10.1016/B0-72-169377-6/50020-4.

R Core Team, 2019. A Language and Environment for Statistical Computing. R Foundation for Statistical Computin. https://doi.org///www.R-project.org/.

Rault, J., 2019. Be kind to others : Prosocial behaviours and their implications for animal welfare. Appl. Anim. Behav. Sci. 210, 113–123. https://doi.org/10.1016/j.applanim.2018.10.015.

Rödel, H.G., Starkloff, A., Bautista, A., Friedrich, A.C., Von Holst, D., 2008. Infanticide and maternal offspring defence in European rabbits under natural breeding conditions. Ethology 114, 22–31. https://doi.org/10.1111/j.1439-0310.2007.01447.x.

Rommers, J.M., Boiti, C., De Jong, I., Brecchia, G., 2006. Performance and behaviour of rabbit does in a group-housing system with natural mating or artificial insemination. Reprod. Nutr. Dev. 46, 677–687. https://doi.org/10.1051/rnd:2006038.

Rommers, J.M., Gunnink, H., Klop, A., De Jong, I.C., 2011. Dynamics in aggressive behaviour of rabbit does in a group housing system: a descriptive study. In: 17th International Symposium on Housing and Diseases of Rabbits, Fur Providing Animals and Pet Animals. May 11-12. Celle, Germany,. pp. 75–85.

Rommers, J.M., Reuvekamp, B.J.F., Gunnink, H., de Jong, I.C., 2014. Effect of hiding places, straw and territory on aggression in group-housed rabbit does. Appl. Anim. Behav. Sci. 157, 117–126. https://doi.org/10.1016/j.applanim.2014.05.011.

Rosenblatt, J.S., 1980. Hormonal and Nonhormonal Regulation of Maternal Behavior : a Theoretical Survey to Cite This Version : HAL Id. hal-00897684. .

Ruis, M., 2006. Group housing of breeding does. In: Coupert, P., Maertens, L. (Eds.), (Eds.), Recent Advances in Rabbit Sciences. ILVO, Melle, Belgium, pp. 99–205.

Schlolaut, W., Hudson, R., Rödel, H.G., 2013. Impact of rearing management on health in domestic rabbits: a review. World Rabbit. Sci. 21, 145–159. https://doi.org/10.4995/wrs.2013.1029.

Seaman, S.C., Waran, N.K., Mason, G., D’Eath, R.B., 2008. Animal economics: assessing the motivation of female laboratory rabbits to reach a platform, social contact and food. Anim. Behav. 75, 31–42. https://doi.org/10.1016/j.anbehav.2006.09.031.

Snow, A.E., Horita, A., 1982. Interaction of apomorphine and stressors in the production of hyperthermia in the rabbit. J. Pharmacol. Exp. Ther. 220, 335–339.

Southern, A.H.N., 2019. Sexual and Aggressive Behaviour in the Wild Rabbit. Published by : Brill Stable URL :1. pp. 173–194. https://www.jstor.org/stable/4532685.

Stoffel, M., Nakagawa, S., Schielzeth, H., 2019. rptR: Repeatability Estimation for Gaussian and Non-Gaussian Data. https://cran.r-project.org/package=rptR.

Surridge, A.K., Bell, D.J., Hewitt, G.M., 1999. From population structure to individual behaviour: genetic analysis of social structure in the European wild rabbit *(Oryctolagus cuniculus)*. Biol. J. Linn. Soc. 68, 57–71.

Szendrő, Z., Gerencsér, Z., McNitt, J.I., Matics, Z., 2016. Effect of lighting on rabbits and its role in rabbit production: a review. Livest. Sci. 183, 12–18. https://doi.org/10.1016/j.livsci.2015.11.012.

Trocino, A., Xiccato, G., 2006. Animal welfare in reared rabbits: a review with emphasis on housing systems. World Rabbit. Sci. 14, 77–93. https://doi.org/10.4995/wrs.2006.553.

Tsutsumi, Y., Hachinoe, Y., Ogura, N., Takahashi, M., 1967. Variations in body temperature associated with reproductive state in the female rabbit. J. Fac. Agric. Hokkaido Univ. 55 (4), 363–381.

Turner, S.P., 2011. Breeding against harmful social behaviours in pigs and chickens: State of the art and the way forward. Appl. Anim. Behav. Sci. 134, 1–9. https://doi.org/10.1016/j.applanim.2011.06.001.

Valuska, A.J., Mench, J.A., 2013. Size does matter: the effect of enclosure size on aggression and affiliation between female New Zealand White rabbits during mixing. Appl. Anim. Behav. Sci. 149, 72–76. https://doi.org/10.1016/j.applanim.2013.10.002.

Varga, M., 2014. Rabbit Basic Science. Textbook of Rabbit Medicine. pp. 3–108. https://doi.org/10.1016/B978-0-7020-4979-8.00001-7.

Vella, D., Donnelly, T.M., 2012. Basic Anatomy, Physiology, and Husbandry, Third Edit. ed, Ferrets, Rabbits, and Rodents. Elsevierhttps://doi.org/10.1016/B978-1-4160-6621-7.00012-9.

Verga, M., Luzi, F., Carenzi, C., 2007. Effects of husbandry and management systems on physiology and behaviour of farmed and laboratory rabbits. Horm. Behav. 52, 122–129. https://doi.org/10.1016/j.yhbeh.2007.03.024.

Zomeño, C., Birolo, M., Zu, A., Xiccato, G., Trocino, A., 2017. Aggressiveness in Group-housed Rabbit Does : Influence of Group Size and Pen Characteristics. 194. pp. 79–85. https://doi.org/10.1016/j.applanim.2017.05.016.

Zomeño, C., Birolo, M., Gratta, F., Zuffellato, A., Xiccato, G., Trocino, A., 2018. Effects of group housing system, pen floor type, and lactation management on performance and behaviour in rabbit does. Appl. Anim. Behav. Sci. 203, 55–63. https://doi.org/10.1016/j.applanim.2018.03.002.